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Genome-wide association studies identify disease mechanisms in age-related macular degeneration

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Separating causal from confounding associations with disease has been a long-standing problem in epidemiology. The situation improved when it became feasible to perform genome-wide association studies (GWAS): genotyping of large case-control cohorts at several million single-nucleotide polymorphisms (SNPs) spread across the genome, in an unbiased screen for association (i.e. with no prior hypothesis). A key factor in their success was the use of stringent statistical thresholds to control for the numerous tests. Since genotypes are randomly distributed at meiosis, predate most traits and remain stable throughout life, they are not generally subject to confounding influences and so have provided robust and causal associations. GWAS also confirmed classical genetic theory stating that the majority of complex traits are influenced by innumerable variants with tiny individual effects. A recent extension of this theory, the “omnigenic” model, proposes that most traits are influenced by variants in a limited set of “core” genes, with direct and biologically interpretable effects, alongside more numerous “peripheral” genes, mostly with very small effects and acting through inter-connected regulatory networks.¹ In fact, peripheral rather than core variants account for most trait heritability because, despite their small effects, there are over 100 times more of them. Peripheral variants lie outside coding regions and individually provide limited insights into trait biology. Indeed most are only detectable collectively. As an example, >100,000 SNPs are estimated to show independent causal effects on human height¹, the vast majority exerting only tiny “peripheral” effects and providing limited biological insights.¹ Given these considerations, what insights have been gained from GWAS in age-related macular degeneration (AMD)?

On page xx, Lores-Motta et al.² describe two new GWAS associations that confirm a core pathway and illuminate AMD pathogenesis. Genetic associations in AMD were amongst the first fruits of the GWAS approach, helped by uncharacteristically strong effects.³ One of these was attributed to a SNP (rs1061170) in the complement factor H gene (*CFH*), causing a Tyr402His substitution³. This may compromise the ability of its product, the soluble glycoprotein complement factor H (CFH), to suppress activation of complement on the surfaces of host (self) tissues while allowing complement to proceed unchecked on foreign surfaces (Fig. 1).⁴ Additional, independent, missense *CFH* SNPs that likely impact on CFH function were also reported. Other AMD-associated SNPs lay in non-coding, presumed regulatory, regions. Rare but more highly penetrant variants of *CFH* were later found in small subsets of AMD subjects. Unfortunately, while GWAS associations are statistically robust and indicative of causal associations, they don’t always indicate the precise nucleotides responsible. This is a particular problem in the *CFH* region, where there is a strong tendency for neighbouring nucleotides to be co-inherited in blocks that are only rarely separated by recombination (this is called linkage disequilibrium).

A dysfunctional complement pathway in AMD was independently supported by immunohistochemistry showing that CFH and other complement components were present in drusen, a hallmark of AMD. Subsequently, other complement encoding genes (C3, factor B, factor I, C9, vitronectin) were associated with AMD in further and bigger GWAS, the

largest of which included >16,000 advanced AMD cases and ~18,000 controls.⁵ Complement pathway variants collectively accounted for about one-third of AMD risk.⁵ When the collective effect of genome-wide SNPs in AMD was partitioned into functional subsets, the most significant contribution came from ~1,300 SNPs in and around genes influencing complement biology.⁶ Together, the results pointed to a causal role for increased complement activation in AMD.

These exciting findings stimulated a high level of translational and commercial activity but uncertainty remains over the relative importance of systemic versus local complement activation in AMD. While most complement genes are strongly expressed by the liver and encode proteins that circulate at quite high levels in the blood, other sources of complement proteins include the cells lying on either side of Bruch's membrane (Fig. 1). This raises the key question of where to target therapeutic intervention. While systemic activation could damage the choriocapillary endothelium, local retinal or choroidal dysregulation might contribute most to retinal pigment epithelial (RPE) damage or drusen formation.

The case for systemic complement activation was strengthened by studies of the "complotype" or joint effects of common functional variants in the *CFH*, *CFB* and *C3* genes.⁷ When combined, these showed up to six-fold variation in complement activation measured *in vitro*. Complotype is thus likely to influence an individual's systemic complement activation. But a similar effect of complotype might be manifested in the eye, depending on the local levels of the various proteins, which remain to be established. Those liver transplant recipients who develop AMD reportedly carry the recipient rather than donor *CFH* Y402H risk allele, suggesting that local complement activation in the eye trumps systemic activation.⁸

Other evidence supporting the primacy of systemic complement activation stems from the finding that complement activation end-products are elevated in patients' blood.⁸ C3d is a stable proteolytic fragment of C3 and the endpoint of successive cleavages beginning with C3 cleavage to C3b, the key step in complement cascade activation (Fig. 1).⁹ C3b's short-lived thioester group either hydrolyses or binds covalently to nearby surfaces. C3b, in fluid phase or on surfaces, is eventually inactivated by cleavage to iC3b and then to C3dg and finally C3d. Surface-tethered C3d can remain resident for an extended time. The ratio between serum concentrations of C3d and C3 is used as a proxy for systemic complement activation.² Note that this ratio takes no account of potentially large amounts of C3d bound to cellular and other surfaces. Hence it reflects C3 consumption and fluid-phase activation but does not report directly on surface complement activation.

Lores-Motta *et al.*² address the role of systemic complement activation by reporting a GWAS of serum C3d/C3 ratios. The authors studied 717 AMD cases and 831 controls in a discovery cohort and confirmed their findings in a smaller replication cohort. No genetic variants outside a cluster of genes on chromosome 1, which includes *CFH* and five protein-coding *CFH*-related genes (*CFHR1-5*), showed significant association with C3d/C3. Of the two independent variants that did show genome-wide significant associations with C3d/C3, one (the strongest) was a coding variant in exon 14 of the *CFH* gene (rs3753396) that did not

change the amino acid sequence. The other was a non-coding SNP (rs6685931) in the *CFHR4* gene. Like other CFH-related proteins, CFHR4 antagonises the action of CFH, thus potentially promoting complement activation¹⁰, although its serum levels are 10-30-fold lower than those of CFH. Interestingly, deletion of *CFHR1* and *CFHR3* is protective in AMD, but this may be due to linkage disequilibrium (see above) with causal variants in the neighbouring *CFH* gene.¹¹ Lores-Motta *et al.*'s report² that a *CFHR4* SNP, also in strong linkage disequilibrium with *CFH* SNPs, is associated with systemic complement activation, is an intriguing one, despite the difficulty of disentangling causal SNPs in the region.

Since a *CFHR4* SNP (rs6685931) and a *CFH* SNP (rs3753396) are both associated with systemic complement activation, the question arises as to whether or not they are also associated with AMD, since this would imply the presence or absence, respectively, of a causal connection. The observed result, namely that rs6685931 is associated with AMD and rs3753396 is not associated, is potentially confusing. However there may be a prosaic explanation for this apparent discrepancy. The *CFH* "risk" allele is at substantially lower population frequency than the *CFHR4* risk SNP. In this case, the study may simply have lacked the statistical power to show association of the *CFH* variant in a relatively small AMD cohort. Moreover, AMD is a more complex trait than the C3d/C3 ratio, so SNP effects on the disease are likely to be smaller and harder to detect. Alternatively, there could, after all, be a disconnect between systemic complement activation and AMD.

This study highlights some key considerations for those developing complement pathway therapeutics. First, while complement activation is not the only genetically influenced pathway in AMD, it is further confirmed as a major player. Second, the association of a *CFHR4* variant with both AMD and increased systemic complement activation merits attention, although teasing apart the causal variant(s) will be challenging. Third, systemic complement activation surely has a role in AMD, but may influence only part of a complex disease process operating on both sides of Bruch's membrane (Fig. 1). Fourth, genetic effects that influence circulating or ocular complement activation may be correlated, in which case easily measured systemic markers such as C3d/C3 would be useful for stratifying patients in clinical trials.

In conclusion, several complement pathway SNPs are uncharacteristically common in the general population, considering that they exert large effects on complement function. This may reflect past evolutionary pressure to resist infectious diseases.¹² This factor has certainly enabled the detection of core genes and causal pathways in AMD, which emerges as a paradigm for using the power of GWAS to elucidate disease.

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Figure legend

Figure 1. Blood-retinal interfaces and exposure to the complement system. **A**, Schematic diagram of the outer retina and choroid showing (italics) five potential sites of complement-mediated damage. **1**, *RPE tight junctions*, form the outer blood-retinal barrier, breached in advanced AMD. **2**, *sub-RPE space*: between basal RPE and Bruch's membrane, becoming evident in AMD due to basal deposits and drusen. **3**, *Bruch's membrane*, a pentalamellar membrane interface between blood and retina. **4**, *fenestrations* in the lining of choroidal capillaries, facilitate access to Bruch's membrane by complement proteins. **5**, *endothelium* of choroidal capillaries, directly exposed to systemic sources of complement. **B**, Factor H suppresses both fluid-phase and surface complement activation. In the absence of inhibition by factor H, C3b amplification leads to conversion of C5 to C5b and formation of the cytolytic membrane-attack complex C5b-9ⁿ. C3d is an end-product of complement activation (*i.e.* conversion of C3 to C3b) following factor H/factor I-assisted inactivation. CFHR4 may inhibit the action of CFH. *The ratio, soluble (serum) C3d/C3 has been used as a proxy for systemic complement activation in a GWAS.

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